

**Interdisciplinary Studies of Methylmercury in
San Francisco Bay and New Almaden
Sediments: Monitoring, Microbial
Associations, Complexation, and Toxicity to
Salmonids**

A. Russell Flegal

Public Comments

No public comments were received for this proposal.

Collaboration Panel Review

Proposal Title

#0309: Interdisciplinary Studies of Methylmercury in San Francisco Bay and New Almaden Sediments: Monitoring, Microbial Associations, Complexation, and Toxicity to Salmonids

Final Panel Rating
superior

Collaboration Panel (Primary) Review

Collaboration:

Will the results of the collaborative effort be greater than the sum of its parts? Is it clear why the subprojects are part of a larger collaborative proposal rather than several independent smaller ones?

above average

The proposal plans to couple its sampling program with that of a larger one (SF Bay RMP) to enable comparisons and integrate new data. The approach is to integrate 4 projects into one proposal to capitalize on complementary data sets.

Interdependence And Integration:

Does the proposal have an example that clearly articulates the conceptual model of each subproject and how they link together as a whole? Are the boundaries of the study plans focused and cohesive, yet well delineated? Is there a plan for potential differences in the stages of subproject completion times? Are there clear plans for analyses and interpretations which seek to identify and quantify relationships among the data collected in various subprojects rather than separate analyses for each subproject?

above average

The proposal describes a clear plan for collection of different, complementary data and using that information to generate a comprehensive understanding of new and existing protocols regarding sediment-derived methylmercury analyses and what factors influence the formation of methylmercury in those sediments. Tasks and boundaries are well defined. The

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advantages to the collaboration are based on quantifying relationships among the data collected in each project.

Project Management:

Is it clear who will be performing management tasks and administration of the project? Are there resources set aside for project management and time given for investigators to collaborate? Is there a process for making decisions during the course of the project? Are there acknowledgments of potential barriers to collaboration and explanations of how team members will overcome barriers particular to their institutions?

superior

Personnel tasks and roles are well defined. (Even back-up personnel are named in case the first person is unable to carry out the task/role.) Coordination is planned through numerous meetings. Statements for dealing with potential problems (as indicated from past experience) are described in the proposal.

Team Composition:

Does the lead principal investigator have successful management history and experience leading collaborative teams? Is it clear that all key personnel are committed to making significant contributions to the project? Do team members have complementary skills?

superior

"Dr. Flegal has administered multiinstitutional grants and productively interacted with each of the principal investigators for several years." It is clear that each person has committed to making their described contribution to the project as these descriptions are very specific in the proposal. Skill are complementary among the group.

Communication Of Results:

Is there a clear plan for comprehensive and cohesive reporting of project progress to the CALFED community?

adequate

Plans for presentation of information: poster/oral presentations, final and annual reports, workshop, and journal

Collaboration Panel Review

articles are stated.

Additional Comments:

A clear, well-written proposal.

Collaboration Panel (Discussion) Review

Secondary reviewer judged it as Superior in almost all categories. It was well written, with complex tasks linking closely together. There was a clear integration and all elements was tied together at the end. Project management allocations were adequate. Team composition is excellent. Communication of high impact products has a strong feedback loop to managers and scientists.

Primary reviewer adjusted Above Average to Superior rating as a result of panel discussion. The primary reviewer felt the proposal contained one of the best management sections of any read.

Technical Synthesis Panel Review

Proposal Title

#0309: Interdisciplinary Studies of Methylmercury in San Francisco Bay and New Almaden Sediments: Monitoring, Microbial Associations, Complexation, and Toxicity to Salmonids

Final Panel Rating
adequate

Technical Synthesis Panel (Primary) Review

TSP Primary Reviewer's Evaluation Summary And Rating:

The authors propose a collaborative study of Hg in the Bay-Delta region by combining studies of field and laboratory protocols for methyl Hg in sediments, identification of sulfate reducing bacterial community structure in relation to MeHg concentrations in sediments, complexation of MeHg in the aqueous phase and a study on sublethal toxicity to larval fish. The individual projects would yield interesting results and the lead PI has experience in Hg cycling in the Bay-Delta region. The proposal lacks a true integration of the separate phases of the study. While clear hypotheses are provided for each individual section, the overall proposal lacks a conceptual model, a key component for other proposals reviewed by this call. It made it quite difficult for reviewers and the lead panelist to determine the clear benefits and outcomes of the interdisciplinary work. A significant portion of the study is devoted to developing a method for determining MeHg in sediments, which should be a part of a valid QA/QC component of a project, not a separate study. Will the different sampling strategies for solid phase MeHg be applied in a pairwise fashion or in a factorial manner? That aspect could be quite important in determining the final recommended protocol. The work on sulfate reducing bacteria is novel and interesting, but it is difficult to understand the direct

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Technical Synthesis Panel Review

links of population structure to MeHg concentrations in sediments. For instance, if MeHg were the result of deposition, rather than production, the interpretation might be in error. Certainly determination of methylation rates in sediments where sulfate reducing bacteria composition work would help. The resin studies to determine the degree of DOC complexation is interesting, yet there is no tie to how the results will be used to address bioavailability in the system. The reliance of MXR as the biomarker for MeHg is risky, since its use rests on a number of untested assumptions (first that it is actually present in fish, second that it is a specific biomarker for MeHg).

Additional Comments:

The authors propose a collaborative study of Hg in the Bay-Delta region by combining studies of field and laboratory protocols for methyl Hg in sediments, identification of sulfate reducing bacterial community structure in relation to MeHg concentrations in sediments, complexation of MeHg in the aqueous phase and a study on sublethal toxicity to larval fish. The individual projects would yield interesting results and the lead PI has experience in Hg cycling in the Bay-Delta region. The proposal lacks a true integration of the separate phases of the study. While clear hypotheses are provided for each individual section, the overall proposal lacks a conceptual model, a key component for other proposals reviewed by this call. It made it quite difficult for reviewers and the lead panelist to determine the clear benefits and outcomes of the interdisciplinary work. A significant portion of the study is devoted to developing a method for determining MeHg in sediments, which should be a part of a valid QA/QC component of a project, not a separate study. Will the different sampling strategies for solid phase MeHg be applied in a pairwise fashion or in a factorial manner? That aspect could be quite important in determining the final recommended protocol. The work on sulfate reducing bacteria is novel and interesting, but it is difficult to understand the direct links of population structure to MeHg concentrations in sediments. For instance, if MeHg were the result of

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Technical Synthesis Panel (Discussion) Review

TSP Observations, Findings And Recommendations:

Interdisciplinary studies of methylmercury in San Francisco Bay and New Almaden sediments: monitoring, microbial associations, complexation, and toxicity to salmonids

The four components of the proposal were recognized as important areas of Hg research. However, the reviewers and the panel all felt that the proposal did not adequately integrate these components into a strong program that addresses bioavailability and uptake to higher trophic levels. The proposal did not follow the required PSP format and did not provide a true conceptual model for the study. Concerns were expressed over the large portion of the study that was devoted to method development for MeHg in sediments. If anything, this section should be a QA/QC portion of the MeHg-sulfur bacteria survey. It is important to determine if MeHg is transported and deposited or whether it was produced in situ. PIs should perform direct measurements of methylation to correlate with the presence of sulfur bacteria. Also, the use of MXR biomarker was questioned, given that it is generally nonspecific and has not been demonstrated to actually occur in salmonids. These concerns contributed to the final ranking of adequate.

Final Ranking: Adequate

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proposal title: Interdisciplinary Studies of Methylmercury in San Francisco Bay and New Almaden Sediments: Monitoring, Microbial Associations, Complexation, and Toxicity to Salmonids

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	Objectives and hypotheses are clearly stated and are internally consistent. The idea is timely and important. Methyl mercury pollution may be an important issue for the San Francisco Bay watershed, and it is necessary to understand the interplay between sediment geochemistry and microbial processes responsible for the mercury methylation. It is also important to understand the bioavailability and toxicity of the methyl mercury.
Rating	excellent

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

Comments	The overall study is justified relative to existing knowledge, though justification varies for the different components of the proposed research. Task I (developing protocols for accurately measuring MeHg in sediments) is important, but it is difficult to believe that an SF Bay monitoring program has been up and running for a while without having first developed such a protocol. Moreover, it is not clear how this
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	<p>task compares to recent publications that deal with this issue (Horvat et al. 2004, Ogrinc et al. 2004, Parker and Bloom 2004); does Task I merely address known problematic protocol steps that have just not yet been determined for San Francisco Bay sediments or does it look at other protocol steps (that have not yet been critically evaluated)? Task II is the most interesting part of the project, and is well justified. Task III is important, but is limited to freshwater (so does not fully articulate with the rest of the project), while it appears to duplicate ongoing research in the PI's lab (for the current proposal, three more sample sites will be added to the ongoing study). Task IV would be justified if it really addressed the issue of mercury bioavailability and toxicity in larval fish, but it is limited to the multixenobiotic resistance mechanism (and its potential as a biomarker of MeHg exposure) which is, at best, only indirectly related to MeHg exposure or toxicity (see comments under "Approach"). The proposal does not contain a conceptual model explaining the underlying basis of the proposed work.</p>
Rating	fair

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	<p>The overall approach, with the four specific tasks, is appropriate for meeting the objectives of the project. However, some tasks do not meet the overall project goals. The overall approach for Task I (development of protocol for measuring MeHg in sediments) is appropriate. However, it is not clear how the researchers will decide what methods are superior. For example, when comparing the results for different sediment pre-treatment methods, is the method that</p>
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Technical Review #1

yields the highest MeHg value the best one? Or is it the method that results in the lowest amount of variability among replicated samples? The time-line for this task mentions three study phases, but these three phases are never clearly described. While ancillary data are collected (as part of the RMP) for the San Francisco Bay samples, it is unclear whether these will be collected (by the researchers) for the New Almaden sites. The approach for Task II seems solid and to be well suited for addressing this task's objectives. The same holds for Task III, with this task's limitation (imposed by methodology) to freshwater conditions. This leaves unanswered the question what changes occur in the MeHg complexation once this freshwater enters San Francisco Bay. The approach for Task IV ("mercury bioavailability and sublethal toxicity in larval fish") fails to adequately address the issues of MeHg bioavailability and toxicity. The approach that is used here focuses on the multixenobiotic resistance (MXR) membrane transport. This is a mechanism implicated in the export of hydrophobic xenobiotics from invertebrates. The hypothesis for this Task is "The MXR transport mechanism is present in embryonic and larval salmonids and is affected by exposure to MeHg; therefore MXR can potentially be used as a sensitive biomarker of salmonids exposure to MeHg". So at best this would develop a biomarker for detecting MeHg exposure; a tool that COULD be used to assess bioavailability. But this would require the following: 1) the MXR system is present in fish, 2) MXR is affected by MeHg (the proposal authors cite two studies showing maternal transfer of MeHg in walleye, then go on to state that the effects of such a transfer on MSX is unknown and that it is plausible that MXR is inhibited in larvae exposed to MeHg - without providing any back-up for this statement). The authors even mention the possibility that MXR is a defense mechanism against MeHg toxicity - in which case one might expect it to be induced by MeHg exposure rather than being inhibited. 3) MXR is affected more by MeHg than by

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	other contaminants or by other environmental variables. There is no evidence that MXR is affected by MeHg, and no information is provided how it is affected by other contaminants. The possibility seems very remote that MXR would be a sensitive biomarker of MeHg exposure. This task has three components: a) developing methods to measure MXR in zebrafish, b) determining whether MXR is affected by exposure to MeHg, and c) detecting MXR in developing salmon. Even if successful, it still does not get to the issue of determining whether MXR is a useful indicator of MeHg exposure in salmon. It is even further away from determining MeHg bioavailability and toxicity.
Rating	fair

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success?
Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	The approach is technically feasible. The likelihood of success varies from high (for some tasks) to low (for e.g. Task IV when it comes to using MXR as a biomonitoring tool and using it to evaluate MeHg toxicity and bioavailability). The scale of the project seems consistent with the objectives and within the grasp of the principal investigator.
Rating	very good

Monitoring

If applicable, is monitoring appropriately designed (pre–post comparisons; treatment–control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	Task I of this project ties in with monitoring done as part of the RMP for San Francisco Bay. The plan is to develop new protocols for MeHg analysis of sediments and to intercalibrate those protocols with current
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	methodologies in the RMP. Critically assessing each of the sampling and analysis steps of a monitoring protocol is very important with respect to the validity of the data generated in the monitoring program, and should have been done prior to the ongoing San Francisco Bay RMP. The project itself does not include monitoring of any restoration projects.
Rating	good

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	Products of value are likely from the project. Results from Task I are important for interpreting results from (and potentially improving methodology for) the ongoing RMP and monitoring projects elsewhere that include MeHg analysis of sediment samples. This assumes that the researchers adequately address the issue of objectively evaluating which protocol modifications are beneficial (see comments made under "Approach"). Tasks II and III should provide important information on the link between MeHg levels and the presence and activity of sulfate-reducing bacteria, and on the role of sulfur ligands in Hg complexation in freshwater systems. Task IV may shed light on the presence of the multixenobiotic resistance membrane transport in fish, but unlikely to contribute significantly to the overall goal of the project.
Rating	very good

Additional Comments

Comments	It is disturbing to see that the PI has listed as a potential reviewer someone (R. Mason) who has closely collaborated with this research group in the recent past! E.g. publication in press: Conaway, Mason, Steding and Flegal, 2005. Earlier publication (2003): Conaway, Squire, Mason and Flegal. The proposal does not follow the general layout for proposals under this RFP, making it difficult to match the proposal with the review criteria. Moreover, the proposal authors failed to present the critical information for their project within a 20-page limit.
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Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	Several of the investigators (especially Russell Flegal and Nicolas Bloom), have a very solid track record in this type of work. While Christopher Francis does not seem to have experience with Hg methylation and sulfur-reducing bacteria, he does appear to have the appropriate background in molecular microbial ecology necessary for task II. The project relies fairly heavily on three graduate students (Conaway, Black, and Langsner), all of which are listed as principal participants and placed in charge of one of the four tasks. This should work fine for Conaway, who is about to graduate and has an excellent publication record for someone at that stage of their career. But Black is only in his third year (no publications), while Langsner is a first year graduate student! Putting a first year graduate student in charge of a substantial portion of an almost 1 million \$ project seems to be overly risky. Based on previous work by the investigators, the support and infrastructure needed to carry out the proposed research seem to be available.
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Rating	good
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Budget

Is the budget reasonable and adequate for the work proposed?

Comments	The budget seems adequate for the proposed research. The overall amount seems on the high side, especially since a substantial portion of the sampling will be done as part of the S.F. Bay Regional Monitoring Plan and since a substantial portion of part III is being done by Black as part of an NSF graduate fellowship.
Rating	good

Overall

Provide a brief explanation of your summary rating.

Comments	The topic of MeHg formation/complexation/bioavailability/toxicity is an important one for the San Francisco Bay area. This proposal aims to combine the various aspects, but success at doing this appears mixed, with definite strong points and weak ones in the proposal. Justifications for the different tasks differ, as does the validity of the approaches. The evaluation of the feasibility of the different approaches also yields a mixed picture. As far as capabilities of the researchers, too much responsibility is assigned to junior Ph.D. students.
Rating	good

Technical Review #2

proposal title: Interdisciplinary Studies of Methylmercury in San Francisco Bay and New Almaden Sediments: Monitoring, Microbial Associations, Complexation, and Toxicity to Salmonids

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	It is difficult to assess goals in parts of the proposal. Individual components of this work are important by themselves. A higher level of integration among project sub-components is lacking, and it is difficult to see how the separate parts fit together into a broader whole. An obvious approach would be to link the work in sections #2 and #3 (role of SRB and complexation) to improving our understanding of Hg levels in higher consumers such as fish, though the links between the processes being examined and bioaccumulation/effects are weak. Making such a link to levels of Hg in the biota would be an easy addition to this project. For example, section #4 does not actually address bioaccumulation (as stated in the general introduction) or even Hg bioavailability (as indicated in the title of section #4). Instead, it deals with a particular biomarker that could be used to measure Hg exposure. These types of inconsistencies make it difficult to assess the actual goals of the project.
Rating	fair

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection

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Technical Review #2

of research, pilot or demonstration project, or a full-scale implementation project justified?

Comments	Much of the individual research proposed is justified. The projects have general implications for our understanding of mechanisms and processes that might affect Hg cycling in the environment. Unfortunately, the links to environmental /biotic Hg levels are not made, though many of the hypotheses imply that such connections are made or will be examined in this study.
Rating	good

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	#1) The approach is quite thorough, and addresses fundamental issues for measurement and monitoring. #2) dsrAB approach seems both appropriate and innovative for addressing the role of SRB in Hg transformation. Investigators adequately address statistical handling of the data. #3) Methods for work complexation seem appropriate, but I do not see how the proposed work actually addresses the hypothesis that the bioavailability and toxicity are limited by organic and inorganic complexation. The link between complexation and toxicity is not there. #4) Developing biomarkers for Hg exposure is an important task, but I don't see how MXR is a Hg-specific biomarker, as is suggested. In field sites such as these which may involve exposure to a suite of contaminants, this biomarker would be for exposure to toxic substances in general.
Rating	good

Technical Review #2

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success?
Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	Some of the approaches are feasible. #1 and #2 are particularly strong. For #3 - are the methods for measuring complexation already developed? From the methods described in the section, it seems as if they are, though one objective listed is to develop the methods to measure complexation. #4 is the most problematic, and I do not believe that the proposed approach will serve as an indicator specifically of Hg exposure in field settings. The approach is still in the very early stage of development, and it is highly uncertain whether this will serve as a useful biomarker. Thus, this aspect of the project falls within the realm of basic research. As such, this part of the proposal is not a good match with CALFED's mission to fund programs on Bay-Delta projects.
Rating	good

Monitoring

If applicable, is monitoring appropriately designed (pre-post comparisons; treatment-control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	The sediment sampling protocol work is of great importance to monitoring. MeHg measurements in sediments may indeed be high sensitive to methodological issues that have not been addressed. Other aspects of the project will provide basic information about processes, but would have less relevance to monitoring of actual ecosystems or Hg levels in the environment.
Rating	good

Technical Review #2

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	There is potential for some important products from this work. The most important would be the rigorous assessment and develop of the sediment sampling protocols. The proposal shows a strong commitment to publication and sharing of knowledge, integration within CALFED, collaboration, science communication, and overall coordination.
Rating	good

Additional Comments

Comments

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	Investigators have strong research track records and as a group, are qualified to carry out the proposed project. The multi-disciplinary and multi-institutional aspect is strong, provided the investigators are able to effectively integrate the diverse aspects of this project. A plan for coordination is laid out in the section entitled coordination, so this aspect has been thought through.
Rating	very good

Budget

Is the budget reasonable and adequate for the work proposed?

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Comments	The budget seems appropriate, considering the overall amount of work being proposed.
Rating	very good

Overall

Provide a brief explanation of your summary rating.

Comments	Overall, some aspects of this proposal are strong, though the integration of these four research projects should be strengthened, such that the whole is greater than the sum of the parts. Also, linkages of the work to the Bay-Delta ecosystem are not strong enough, and many aspects of the work are basic research.
Rating	good